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Previews

The 3D Genome Shapes Up For Pluripotency

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Chromosome folding has been long associated with gene expression. Articles in *Cell Stem Cell* and *Cell* by Apostolou et al., (2013), Phillips-Cremins et al., (2013), Wei et al., (2013), and Zhang et al., (2013) uncover chromatin interaction networks linked to the establishment or maintenance of pluripotency and identify some responsible factors.

The promise of stem cell therapies has been profoundly advanced by the seminal finding that differentiated cells can be reprogrammed to pluripotent stem cells by overexpression of a cocktail of transcription factors (Takahashi and Yamanaka, 2006). However this process is very inefficient, and understanding the barriers to reprogramming has become an intense area of study. The 3D organization of the genome is correlated with transcriptional control; for example, chromatin loops bring enhancers into physical proximity with their gene targets (Kagey et al., 2010) and coregulated genes occupy shared nuclear foci, enriched in key transcription factors (Schoenfelder et al., 2010) specifically in the cell types where the genes are expressed. Four recent studies have analyzed the chromatin interactions with key pluripotency genes in mouse and human pluripotent and differentiated cells, and they have uncovered networks of interactions that are specific to embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). One of the recent studies showed that binding of overexpressed OCT4 and NANOG at gene loci was identical between human iPSCs and unreprogrammed cells from the same experiment, but that enhancer-promoter loops within *OCT4*, *SOX2*, and *NANOG* loci were specific to iPSCs, concomitant with transcription from these endogenous loci (Zhang et al., 2013). Looking more systematically, two groups assessed chromatin interactions genome-wide with the pluripotency genes *Nanog* or *Oct4* (Apostolou et al., 2013; Wei et al., 2013), and another simultaneously screened the chromatin conformations around several key loci (Phillips-Cremins et al., 2013) in mouse ESCs and their differentiated counterparts. Again, a set of enhancer-promoter loops and longer-range (over several megabases or across different chromosomes) gene coassociations were unique to pluripotent cells or intermediates being successfully reprogrammed to iPSCs, despite equal binding profiles of the overexpressed transcription factors.

Because gene-centric chromatin interactions frequently correlate with expression of a given gene (Kagey et al., 2010; Schoenfelder et al., 2010), an open question is whether such genomic topologies are a functional cause or a mere byproduct of transcription. Time course studies of the gain or loss of pluripotency-linked chromatin interactions during iPSC generation (Wei et al., 2013) or ESC differentiation (Apostolou et al., 2013) found detectable changes in the chromatin interactions days before transcriptional changes and differentiation phenotypes were observed. Although these data suggest a causal link between chromosome contact establishment and gene activation, earlier experiments showed that artificially induced enhancer-promoter looping was able to stimulate weak transcription of the beta-globin gene in erythroid cells lacking a hematopoietic transcription factor (Deng et al., 2012), indicating that a permissive genome topology is required but not sufficient to induce transcriptional activation. Formation of the transcriptionally permissive chromatin interactions with pluripotency gene loci appears to distinguish the small number of cells able to be reprogrammed to iPSCs from the nonprogrammable cells (Zhang et al., 2013), suggesting that this may form the "epigenetic barrier" to pluripotency. Similarly, activation of beta-globin by a heterologous enhancer inserted on a different chromosome is restricted to a small number of "jackpot" cells in the population (Noordermeer et al., 2011). Although these data were generated using two artificial systems, they suggest a model where chance chromatin interactions allow transient or inefficient transcriptional activation by bringing together a hub of regulatory factors (Figure 1). Progressive stabilization of these interactions and/or additional factors then commit the gene to efficient transcription, perhaps creating a sufficiently permissive environment to allow transcription to occur in the absence of the initiating chromatin interactions (Wei et al., 2013). The detection of pluripotency-gene-linked chromatin interactions specific to partially reprogrammed intermediate iPSCs (Apostolou et al., 2013) is consistent with the idea of a progressive search for a functional genome configuration. It will be interesting to see if such a model can explain gene expression control in normal differentiation and how it would differ for genes subject to more acute transcriptional changes, such as the immediate early genes upon mitogenic stimulation.

Previous research has implicated several protein factors to be responsible for chromatin interactions. Protein-protein interactions among transcription factors bound to promoters and enhancers stimulate chromatin looping (Deng et al., 2012) and longer-range coassociations (Schoenfelder et al., 2010), and more general, non-cell-type-specific factors have also been implicated in genome folding. These include the insulator-binding protein CTCF (Splinter et al., 2006), the cohesin complex that mediates sister chromatid cohesion (Kagey et al., 2010), and the transcriptional coactivator complex, Mediator (Kagey et al., 2010). Again, demonstrating a causal relationship between binding of these proteins and

formation of chromatin loops has been difficult (Deng et al., 2012). Collectively, the four recent studies shed further light on the interplay between transcription factors and the "architectural" proteins in establishing functional chromatin interactions. Phillips-Cremins et al. (2013), in the most detailed analysis of genome folding around several pluripotency gene loci, reveal that chromatin loops are prevalently formed around binding sites of the architectural proteins cohesin, CTCF, and/or Mediator, with significantly less contribution from the binding sites of the pluripotency transcription factors Oct4, Sox2, and Nanog. Interestingly, by comparing ESC and neural precursor interaction profiles, the authors were able to distinguish two general classes of chromatin loops: ESC-specific enhancer-promoter loops, coinciding with cohesin/Mediator binding, and often transcription factor binding, but without CTCF; and larger, constitutive loops coinciding with CTCF and cohesin binding sites, proposed to play a more fundamental architectural role in chromosome folding. The other three articles highlighted here studied the interplay between transcription factors and architectural proteins in more detail. Knockdown of pluripotency transcription factors, for instance Klf4 (Wei et al., 2013) or cohesin or Mediator (Apostolou et al., 2013; Kagey et al., 2010; Zhang et al., 2013), induces differentiation of ESCs or impairs reprogramming to form iPSCs. However, whereas transcription factors were able to bind in both pluripotent and nonreprogrammed cells, the binding of cohesin and/or Mediator was exclusive to cells with a "pluripotency competent" genome configuration (Wei et al., 2013; Zhang et al., 2013). These findings suggest that the epigenetic barrier to pluripotency may be the establishment of permissive chromatin interactions by recruitment of cohesin and/or Mediator (Figure 1), which in some cases may be mediated by direct protein-protein interactions with sequence-specific transcription factors (Wei et al., 2013). While indirect effects, such as cell cycle perturbation on cohesin knockdown, cannot be excluded when interpreting these experiments, this combined body of work suggests an attractive mechanism whereby stochastic permissive chromatin interactions can be stabilized by architectural proteins, allowing nuclear programming during differentiation or establishment of pluripotency. Within this context, the possibility that interactions between pluripotency factors or architectural components may induce posttranslational modifications in order to stabilize pluripotent chromatin configuration remains to be considered.

As cohesin and Mediator do not have sequence-specific binding properties, understanding their recruitment to genomic locations other than known binding sites for the pluripotency transcription factors will also be important to understand the modulation of nuclear architecture. Transcription factors beyond those studied here may generally function by recruitment of these factors or architectural proteins yet to be discovered. Moreover, it will be important to investigate the role of the multiple pluripotency transcription factor binding sites that have not been shown to coassociate with architectural proteins and do not participate in chromatin loops. They may either be involved in loops at cell states that have not yet been investigated, or they may function in different mechanisms to maintain pluripotency. Finally, these studies highlighted gene loop formation for transcriptional activation, but programmed gene loops may be equally important in order to mask differentiation-specific genes in ESCs or during reprogramming to iPSCs, an issue that remains open for future studies.

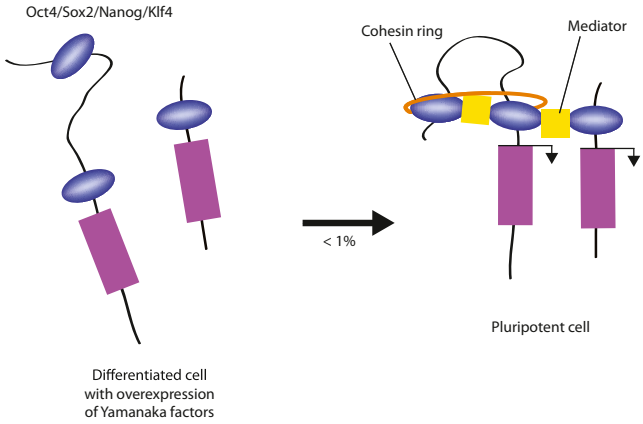

Figure 1. Model for the Role of Chromatin Interactions in Mediating Pluripotency

Overexpressed Yamanaka transcription factors (blue ovals) bind to motifs at the promoters and enhancers of pluripotency genes (purple rectangles) in differentiated tissues (left panel), but very few are reprogrammed to iPSCs. Chance chromatin interactions between enhancers and promoters, and between pluripotency genes, seem to be required for reprogramming (right panel), and are reinforced by further recruitment of the "architectural proteins" Mediator (yellow squares) and cohesin rings (orange circles), providing a permissive environment for transcriptional activation of the pluripotency genes.

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